ANAEROBIC DIGESTION OF FRESH RIPE BANANA PEELS

By

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A THESIS PRESENTED TO THE GRADUATE SCHOOL
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I dedicate this thesis to my loved wife, Marie Carmel J. Saint-Phard, in acknowledgment for her entire devotion to my success and well-being. Without her help, her incentive, and her company I would hardly have been able to satisfy family responsibilities and the obligations of graduate studies.

This thesis is also dedicated to my wonderful children, Jerry and Cassandra Saint-Phard, as a permanent reminder that the road of honesty, character, hope, faith in God, ambition to learn, respect for the moral values is the road I wish they follow. This is the road to success, the road to happiness.

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Chairman: Ross D. Brown, Jr.
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Many useful products might be derived from about 24 million tons of banana wastes that are generated annually throughout the world. Few reports exist on the conversion of banana wastes to biogas or methane. In this study, fresh ripe banana peels were used to produce biogas. The use of (a) gas chromatography to quantify the methane produced, (b) commercial enzymes (cellulase and pectinase) for pre-treatment of the peels prior to anaerobic digestion, and (c) HPLC to identify soluble sugars supported some technologically significant conclusions. Banana peels, either fresh or dried, have the same potential yield of methane indicating that no loss of volatile solids due to

drying the peels occurred. Thus, the peels can be stored in a dry condition prior to digestion. The efficiency of conversion of the peels into methane ranged from 50 to 55% of theoretical estimate. Enzyme-pretreatment of the peels enhanced methane yield by 15 to 20%. Ultimate methane yields, after correction for the contribution of the enzymes to methane production, were not affected. Rather, the principal consequences of enzyme-pretreatment were a shorter lag period during the bioconversion and an increase of 50 to 100% in the rate of methane formation. Pectinase, containing some cellulase activity, was found to be effective for pretreatment of banana peels (of which pectin is a major component) submitted to the anaerobic digestion process.

CHAPTER I
INTRODUCTION

Energy and Environmental Issues

Global trend

For the last two decades, energy and environmental concerns universally have become two fundamental issues which have retained most politicians' attention and which have preoccupied the common citizen's consciousness. Those concerns are partly due to such an ample dissemination of information by the mass media worldwide regarding the adverse consequences that the abuse of both resources might have on mankind's general welfare in the near future. A call to envision and implement remedial approaches is made in order to avoid an actual crisis while establishing, once for all, directives aimed at preventing any subsequent recurrence of this alarming situation.

Energy and the environment are connected in various ways: the quality of life available to mankind will ultimately depend on the degree and type of interactions between both. Energy consumption and status of the environment are two parameters which are used to assess the

development stage of a particular country, with developed countries having both the highest per capita energy consumption rates (estimated to be between 100 and 300 GJ/h.y (1)), and also a relatively cleaner and safer environment.

The influence of geopolitical interests on energy trade can serve either to ally other nations resulting in friendly international relations, or to alienate nations to the point of serious confrontations that can lead to a declaration of war. What is true for international energy trading policies, also holds for environmental accords, but in the latter case, the concerned parties usually are neighboring countries with a common border like the United States and Mexico or Haiti and the Dominican Republic.

Development and progress, ultimate aspirations of mankind, will remain a continuous process in spite of the advocacy of opposing views which desire to maintain a status quo by arguing that development and progress are major contributors to energy and environmental problems. Others consider these energy and environmental problems a result of the population explosion of the last twenty years. This explosion has provoked considerable pressure on land, a limited resource, and consequently, a distortion of the ecosystem.

Alternative sources of energy

Energy is derived from the environment. Its traditional sources are coal, natural gas, and petroleum which are considered to be nonrenewable. During the extraction and, more precisely during the conversion process, large quantities of toxic materials are discharged into the environment as pollutants. The conservation of the environment demands that pollution be controlled by focussing on other so-called renewable sources of energy, such as solar energy, nuclear fusion and bioenergy. Economics, convenience and technology are the factors which will determine which new source(s) will predominate, but it is already believed that the option of choice for the majority of developing countries is bioenergy. In that perspective, fuel from biomass and in particular, anaerobic digestion, the subject of our research, are quickly gaining popularity and adaptability among those countries (2). Any type of industrial, municipal, agricultural or domestic wastes as well as crop and wood residues can be accomodated to anaerobic digestion. Concerning the food industry, some studies on anaerobic digestion of wastes from the dairy, fruit and vegetable, fish and shellfish, fermentation and meat processing plants have been reported (3). The relevance of anaerobic digestion to the food industry can be related to the facts that (1) the food industry is considered as a major pollution

causing sector by dumping huge amount of untreated wastes into the environment, (2) many characteristics of waste streams from the food industry make them amenable to bioconversion; they are usually low in nitrogen, high in carbon, high in biochemical oxygen demand (BOD), and are easily fermentable, (3) the food industry must comply with legal constraints imposed by regulatory agencies for pollution control and environmental protection, and (4) anaerobic digestion can be used as a source of process energy. Very few studies exist regarding anaerobic digestion of banana wastes, and the peels have been considered to be hardly biodigestible. The objective of this work has been precisely to investigate the technical feasibility of converting banana peels into energy in the form of methane. The study was carried out at the laboratory level by performing the biological methane potential (BMP) of this substrate. Results obtained point the way to further development of this process.

CHAPTER II
REVIEW OF LITERATURE

Bananas

The banana is so familiar that, a priori, any information regarding this fruit might be assumed to be trivial or commonplace. Actually, "the banana is a remarkable fruit not only because, to this day, it is the only tropical fruit ever to have scaled the rarefied heights of total consumer recognition, but also because its cultivation anf marketing are so totally different from those of any other. Furthermore, the banana is an item of major economic and hence social importance" (4, p. 127).

Botany. The varieties of bananas are divided into two practical categories: the edible fruit or dessert banana, and the vegetable or cooking banana. In the United States the former is simply called banana, and, the latter is referred to as plantain. Bananas and plantain belong to the family Musaceae of the order Scitamineae. Musaceae is divided into two genera: 1) the genus Ensete, composed of monocarpic herbs which bear no edible fruits and 2) the genus Musa, which contains five sections, Australimusa, Callimusa, Eumusa, Rhodochlamys, and Ingentimusa. Australimusa and Eumusa are the only

two sections which produce edible fruit. Eumusa is far the biggest and geographically most widely ranging section of the genus Musa (5). Nearly all edible banana cultivars of the Eumusa section, it is believed, have derived from the hybridization of two wild members of this section, the diploid M. acuminata (AA) and M. balbisiana (BB). The hybridization can be within or between those species resulting in a triploid cultivar (6). Gros Michel and Cavendish banana cultivars, both triploid (AAA), consecutively have dominated the world banana trade (7). Cavendish's "reign" began some thirty years ago, and this cultivar thoroughly superseded Gros Michel cultivar which was very susceptible to the devastating Panama disease (Fusaril wilt) (8).

Horticultural aspects

Most bananas are grown in the tropics between 20° N and S latitudes, and in the subtropics between 20° and a little beyond 30° N and S (8). In order to achieve superior yield and a product of good quality, the cultivation of export banana is made under optimum conditions. For the tropics, where irrigation is not needed, these conditions are; monthly minimum average temperature of 21°C, and from 7 months to a year average rainfall of at least 150 mm. All subtropic areas must be irrigated, and they are characterized by from 5 months to a year average rainfall of less than 100 mm and by 3 to 5 months of minimum average temperature of 17°C. For both the tropical and

subtropical areas, the alluvial soils, which represent 8% of the tropical soils, agriculturally are the most important and productive (8).

Nutritional and therapeutic values of bananas

More than its nutritional values, it is banana's superior organoleptic qualities that rank it as the most consumed fruit in the world (9). The suave melting texture of a fully ripe banana associated with its distinctive mellow flavor makes a delicious combination. Bananas contain no fats, no cholesterol and no salt, and provide 79 cal/100 g of ripe pulp (10). Hence, bananas are an ideal answer to the actual American nutrition-mania. Bananas are very nutritious, they represent a good source of minerals, being rich in potassium (400 mg/100 g of pulp) magnesium and phosphorous and are a fair source of iron and calcium (10). Bananas also constitute a virtually complete source of vitamins, and are relatively rich in ascorbic acid and vitamin B_6. That makes bananas a frequently selected component in the diet as it is calculated that bananas make a significant contribution to the United States per capita availability of several vitamins (11).

Some widely recognized therapeutic values of bananas are: 1) in the treatment of peptic ulcer; 2) for obese and geriatric patients as the main source of calories; and 3) more importantly, in the treatment of celiac disease in children. Celiac disease is a condition of chronic

intestinal malabsorption. This disorder results from hypersensitivity to the gluten of wheat or rye, and it is treated by use of a completely gluten-free diet. Bananas apparently function as a source of sugar and gluten-free starch with a low content of soft fiber, which is well tolerated in this condition (11).

Banana utilization

Banana is primarily consumed fresh since it is a very perishable fruit whose consumption or utilization can not be delayed once the fruit is fully ripe. The ripening process is very fast at ambient temperatures and can not be stopped. This aspect of the biochemistry of the banana has been the object of various investigations (10, 12). The ripening process may be retarded by conserving unripe mature bananas in an atmosphere of 90% relative humidity at $15^{o}C$, under which conditions respiration is suppressed and the evolution of ethylene is lower than 0.1 ppm. The fruit then can be ripened at will artificially by applying ethylene gas at a rate of 1,000 ppm (1 liter of ethylene per m^3 of room space) when pulp temperatures are between 14 and $18^{o}C$ (12). Since ethylene is explosive at a concentration of 3% in air, it is preferable to use a mixture of nitrogen with 5% ethylene. In this case, 20 liters of the mixture is used per m^3 of room space (13). Nevertheless, no means exist to effectively maintain the fruit in a desired stage of ripeness. The control of the process of ripening is

ultimately related to the role of ethylene in the tissue and to the autocatalytic formation of this hormone during the early phase of the climacteric (14). Therefore, conservation of the fruit is of importance. The principal industrial methods of conservation are canning, drying, freezing or fermentation. Commercially, the main processed product is banana puree which consists of aseptically canned ripe pulp with no preservatives or sugar added. The next most important canned product is sliced bananas in heavy acidified syrup (30^{o} Brix, pH 4.4) (15).

Utilization of banana waste

Waste bananas comprise those that can not be used for human consumption as such, either because they are overripe or because they are undergraded for the appearance to the consumer, those that are rejected by the export firms according to their quality standards, and those lost in the field due to climatologic adversities. No exact figures exist, but it is estimated that those wastes can amount up to 40% of total production within a country or region (16). Since there is no significant degradation of the components of the banana at this stage, the fruit still represents a valuable resource. Waste bananas can be employed in at least three forms: whole banana, pulp or the peels. Whole waste bananas are usually fed directly to animals; in certain instances they are supplemented with protein-rich by-products (17, 18), or fermented (19, 20) to upgrade

their nutritional value as animal feed. The present author has developed such a product from Protein Enrichment of Waste Banana by Solid-State Fermentation (21). The pulp, mostly simple sugars, can be fermented for beer, wine or vinegar production. Other valuable products such as microbial growth media, single cell protein (SCP), juice and candy products can also be obtained (22).

Economics

The socioeconomic importance of bananas to most Latin American countries, the Philippines and some countries of West Africa resembles in various aspects that of petroleum to the OPEC (organization of countries exporting petroleum) members. There exists an international organization of countries exporting bananas (UPEB), whose members are: Colombia, Costa Rica, Dominican Republic, Guatemala, Honduras, Nicaragua, Panama and Venezuela. Goverments' involvement in the banana business is broad since taxes levied on banana exports are for many of those nations the principal source of revenue. Activities related to banana trade provide employment to up to 37% of the population in most of those countries, and except for Venezuela and the Dominican Republic, banana is the main source of foreign exchange for the UPEB members. Table II-1 gives the volume of banana exported for 1984 to 1987. It can be observed that Ecuador, a non UPEB member, is the world's largest banana exporting-country with a yearly average

Table II-1. Volume of Exported Bananas 1984-1987 (in 1000t)

Country	1984	1985	1986	1987
Colombia	921.0	775.3	857.0	912.5
Costa Rica	937.5	803.6	882.3	942.5
Guatemala	260.5	318.6	331.2	327.0
Honduras	829.9	868.4	800.0	876.5
Nicaragua	82.9	90.0	92.0	72.0
Panama	654.6	685.0	585.9	680.2
Venezuela	3.0	3.0	3.0	3.0
Belize	10.6	9.7	13.1	20.0
Brazil	103.2	105.3	101.2	83.0
Ecuador	971.6	1207.9	1365.9	1381.0
Mexico	35.9	42.0	75.2	83.0
Philippines	799.7	789.3	855.7	775.0
Others	1263.1	1274.3	1350.8	1443.9
World Total	6873.5	6972.4	7313.3	7516.6

Source: International Fruit World (23).

of 1,231 thousand tons, followed by Costa Rica with a yearly average of 891 thousand tons of exported bananas. On average, the world production of bananas amounts to 45.6 million tons, but only 7.5 million tons are traded internationally every year. Thus, most of the total production is destined for local consumption. Brazil is the world's largest producer of bananas with a production of 7.8 million tons of bananas per year, but it exports less than 1% (23). Exports of bananas are characterized by a high degree of concentration with more than 70% of world export supplied by 7 countries. Production and marketing are also concentrated in 5 transnational organizations. The United States is the largest banana importing-country; an average of 2,250 thousand tons per year enters its ports. This figure represents 1/3 of the total world export. The consumption of bananas in the United States averages 9.2 kg per capita (24).

Banana Peels

Characteristics

The main biological function of the peel is to provide the endosperm (pulp) with a safe and controlled environment. The peel constitutes a thick envelope that protects the contents against insect attacks and microbial contamination. One author refers to the peel as "a unique dust and microbial proof wrapper" (25, p. 2). The percentage by

weight of the fruit represented by the peel varies from 80, 40 and 30% for mature, ripe and very ripe banana respectively (26). One parameter which is used to evaluate the extent of the ripening process, and, the acceptability of the fruit by the consumer is the peel appearance. In the banana processing industry, a quality control chart similar to the one presented in Table II-2 is used for selecting and, indeed, for pricing the fruit based on the number of dark 'dots' present on the surface of the peel.

Composition of banana peels

The peel of banana is unique in that it contains an exceptional level of amines. Dopamine occurs at a very high concentration in banana peel and is also present in the pulp. It is the primary substrate in enzymatic browning (27). The concentration of dopamine, rather than that of polyphenoloxidase responsible for its conversion to pigment, appears to govern the rate of browning of bananas (28). During ripening, the major changes in peel composition are: 1) a decline in the chlorophyll content from 50 - 100 ug/g fresh weight to almost zero; 2) hydrolysis of half of the starch to sugars; and 3) a decrease in active tannin to one-tenth of its original value in the green fruit (29). A significant feature of banana peel is a high level of tannins, complex polyphenolic substances characterized by their singular astringence. The

Table II-2. Grading of the Banana Fruit Based on the Appearance of the peel. Grades Over 4 Represent Ripe Bananas.

Grade	Appearance of the Banana Peel
1	Green
2	Green with a trace of yellow
3	More green than yellow
4	More yellow than green
5	Only a green pedicel remaining
6	All yellow
7	Yellow flecked with brown dots

concentration of tannins was found to be much more higher in perennial plants than it is in the other categories (i.e deciduous or evergreen) (30). This feature has been also related to the fact that perennials are most resistant to microbial attacks (31). Some botanists believe that the high levels of tannins in the leaves as well as in the peel of the fruit of banana, a perennial plant, might serve to protect them against herbivores and to act as an antimicrobial agent (32). The total amount of tannins remains nearly constant during ripening; the loss or reduction of astringency is rather associated with a change in the chemical form of the tannins (33). Tannins exist in banana as either "free" or active tannins which impart a strong bitter taste to the fruit or "bound" tannins or "vegetable tannates" which are insoluble and supposedly inert and which have little or no effect on palatability. Table II-3 shows the change in active tannins during ripening. The decrease is much more in the peel than in the pulp which contains, of course, much less active tannins.

Analysis. Proximate and chemical analysis of banana peel are reported in Tables II-4 and II-5 respectively. The ash (mineral) composition is given in Table II-6. Banana peels contain more protein and more fat than the pulp. A chromatographic analysis of the free sugars of banana peels reveals a total sugar content of 14.6% of the dry weight with the following sugar distribution:

Table II-3. Changes in Amount of "Active" Tannin in the Pulp and Peel of Bananas During the Ripening Process Expressed as Units per 100 Grams of Tissue.

Days	Fruit Condition	Pulp	Peel
0	Green	7.36	40.5
1	Green	8.01	34.0
2	Green	7.57	28.3
3	Green	4.30	25.4
4	Green	5.02	25.9
5	Coloring	4.30	16.5
6	Coloring	3.87	18.1
7	Coloring	1.95	11.2
8	Eating-ripe	2.84	4.6
9	Eating-ripe	1.99	4.7
10	Over-ripe	2.00	4.5
11	Over-ripe	1.32	3.5

Table II-4. Proximate Analyses of Fresh Ripe Banana Peels

Composition	Per Cent
Starch	2.1
Total Sugars	4.9
Pectin	1.29
Fat	2.66
Crude Fiber	1.93
Ash	2.08
Water	78.80

Source: National Institute of Science and Technology, Manila Cited in (34).

Table II-5. Chemical Composition of Dried Ripe Banana Peels After Sun-drying[a] and Oven-drying[b] Processes (in % Total Weight)

Component	Sun[a]	Oven[b]
Carbon	9.57	
Nitrogen	1.28	1.14
Phosphorous	1.32	
Potassium	4.85	
Ash	12.50	10.76
Volatile Solids	56.99	87.70
Water	13.49	1.39

Sources: [a] Reported in "Utilization of Food Waste Materials for Energy, Food and/or Animal Feeds Production. Biogas From Dried Banana Peelings," (34).

[b] As determined in the present work.

Table II-6. Ash Mineral Composition of Fresh Ripe Banana Peels

Component	Per Cent Dry Basis
K_2O	52.23
Na_2O	2.72
CaO	1.24
MgO	2.17
Fe_2O_3	-
Mn_3O_4	-
P_2O_5	2.59
SO_3	4.52
SiO_2	5.44
Cl	14.60

Source: Carmen Maria Mesequer Guesada (35).

fructose 19%, glucose 23%, and sucrose 56% (34). The fiber content of banana peels is estimated to be 13% on a dry basis and it is composed of lignin 60%, cellulose 25%, and hemicellulose 15% (36).

Banana peel utilization

Since the ratio (w/w) of pulp to peel for mature ripe banana is approximately 1:1 (37), the total world production of banana peels is roughly 24 million tons per year, a vast amount of biomass. Most of this is disposed of in one or two forms: as household domestic garbage, and as by-products of processing factories. In fact, one major banana processing company estimates that about 20,000 tons of banana peels are discarded annually from its plants in South America (38). For the banana puree plants of United Brands Company in Honduras and Panama the amount is about 16,000 tons annually (38). Similar numbers are reported for Gerber Food of Costa Rica (39); and, though no statistics are available, in addition it can be estimated that more than 15,000 tons of peels are discarded annually by the 7 largest banana processing industries in the United States.

Traditionally, banana peels have been used by the small farmer as an animal feed, but on a commercial basis this application of the peel is not widespread. Banana peel alone is not a good animal feed; protein and energy as well as vitamin and mineral supplementations are

required. Attempts to upgrade the feeding value of banana peel include two procedures generally: mixing the peel with other protein-rich by-products or fermenting the peel (40 - 44). Because of the significant quantities of triterpenoids and phytosterols that exist in banana peel (37), it has been considered as a potential source of steroids. Useful application of the peel wax can be found in the manufacture of polish for shoe or for furniture. The most important potential use for banana peels thus far suggested, is in the energy sector. Recently, banana peels have been recognized as an exploitable source for production of low-cost energy. Its utilization as a substrate for ethanol production has been investigated in India (45). Biogas production from banana peels in combination with plant leaves has been studied (46). The principal objective of this present work is to study the biochemical methane potential (BMP) of ripe fresh banana peels solely.

Anaerobic Digestion: Biogas

Anaerobic digestion is a prominent domain of biological waste treatment (BWT). BWT is defined as a process that relies on the utilization of living organisms, parts or products derived from them, to transform the waste organic materials into useful products. When energy is obtained in the form of methane, the process is referred to as anaerobic digestion or bioconversion, which ultimately converts these

materials into a biogas consisting of methane (50 - 70%), carbon dioxide (25 - 45%), and small amount of hydrogen, nitrogen and hydrogen sulfide (47). A secondary benefit associated with the production of methane is the stabilization of the waste which no longer pollutes due to substantial reduction of its biochemical oxygen demand (BOD). Since the production of methane gas is usually the principal objective of the anaerobic process, biogas has become the practical name used in the literature to describe the biological nature of the gas obtained by the mentioned process. BWT might represent a bright glowing focus in the cloudy future of Biotechnology, to paraphrase Dr. Gaden* who assessed the general perspective for biotechnology as: "distinguished past, cloudy future".

Biogas possesses at least five features which make it an appropriate renewable source of energy for developing countries. First, biogas production is considered to have the lowest financial input per kilowatt-hour (kwh) of output, and it is one of the most "mature" technologies in terms of years of use and number of units installed. Second, biogas has the potential to alleviate deforestation, one of the most pressing problems in developing countries actually. Third, a very special advantage of biogas is that biogas

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*Professor E.L. Gaden,Jr., Dept. of Chemical Engineering, University of Virginia - General Foods Fund Seminar, Nov.16, 1989, Food Science and Human Nutrition Dept.,IFAS University of Florida.

mimics natural environmental cycles; such nutrients as nitrogen, phosphorous, and potassium are conserved in the process and can be recycled back to the land as fertilizer in the form of a slurry. The carbon cycle is represented by the conversion of organic carbon from biological sources to carbon dioxide (CO_2) and methane (CH_4) in the biogas process which, therefore, is not contributing to net atmospheric CO_2 production and global warming, as do fossil fuels. Fourth, because animal manures and night soil are digested in the biogas process, the environmental incidence of pathogens is reduced considerably, resulting in a notable improvement in public health. Fifth, biogas is a very versatile technology, which can utilize a large variety of feedstocks, and which is independent of environmental and social milieus' considerations. Furthermore, biogas is a clean-burning fuel which is convenient for domestic use (48).

Microbiology

Biogas production is a simple, natural biological process. Its fundamental principles consist in the degradation of organic materials under anaerobic conditions to methane and carbon dioxide (Figure II-1). In contrast to earlier descriptions of the anaerobic digestion process as a two-stage process consisting of an acid-forming stage and a methane-forming stage, a current, more satisfactory interpretation as a three-stage, four-step process was adopted few years ago (49, 50) for this fermentation which

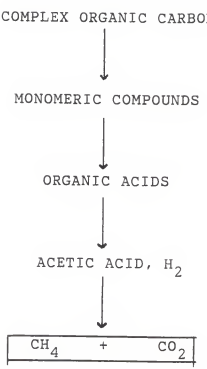

Figure II-1. Biogas Production From Complex Organic Molecules (Simplified Metabolic Routes of Carbon).

is also called methanogenesis (Figure II-2). The three stages are represented by the hydrolysis and liquefaction of the organic matter, the acetogenesis and fermentation of the hydrolyzed products, and the methanogenesis stage proper. The first stage in Figure II-2 depicts the breakdown of the, often insoluble, complex organic matter into simpler, soluble molecules. This involves mainly the enzymic depolymerization and biological conversion of cellulose to sugars, alcohols, peptides, amino acids and fatty acids. The process is initiated by the action of extracellular enzymes released by the microbial flora present in the waste materials. In the second stage, the soluble molecules act as substrates for the fermenting acid-forming anaerobic bacteria to form volatile organic acids, carbon dioxide and hydrogen. Further fermentation by proton-reducing bacteria may be required. Finally, in the methanogenesis stage, the acetic acid and hydrogen produced directly or following fermentation in the preceding stages are converted to methane and bicarbonate (HCO_3^-). Hydrogentrophic methanogenic bacteria then reduce the bicarbonate with hydrogen to form methane (51).

Microorganisms. Two general categories of bacteria are necessary for the methanogenesis process. The nonmethanogenic anaerobic bacteria such as Clostridium, Bacillus, and Micrococcus, responsible for the conversion of the organic

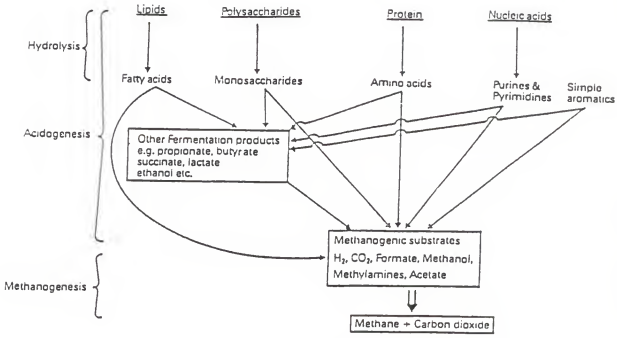

Figure II-2. Theoretical Stages of the Methanogenesis Fermentation.

Source: Samir and Zaborsky (47).

feedstock into simple compounds, and the methanogenic bacteria which convert these compounds into biogas. The latter are strict anaerobic microorganisms comprising four genera: 1) Methanobacterium, a nonspore-forming rod; 2) Methanobacillus, a spore-forming rod; 3) Methanococcus, a nonspore-forming coccus; and 4) Methanosarcina, a nonspore-forming coccus occuring in packets of eight (52). Between the nonmethanogenic and the methanogenic bacteria there exist a syntrophic relationship, their metabolic activities are so coordinated that they benefit mutually one another. This phenomenon involves "interspecies H_2-transfer" referring to the uptake of hydrogen by the methanogens maintaining then a low hydrogen pressure in the milieu and permitting the acetogenic reactions to take place. Otherwise, the overall methanogenic reaction is not thermodynamically feasible (53). The oxidation of H_2 and reduction of CO_2 to CH_4 as well as the aceticlastic dissimilation of acetate to CH_4 and CO_2 each constitute a biochemical system unique to the methanogens. These metabolic capacities of the methanogens require unique enzyme co-factors such as Coenzyme M (CoM), coenzymes F_{420} and F_{430} as a group, and coenzymes F_{350} and F_{340} as another group called the methylpterins (54). All those co-factors are involved in the terminal steps of the conversion of CO_2 to CH_4. Other peculiarities of the methanogens are that they can use only a limited number of

compounds such as hydrogen, carbon dioxide, formic acid, methanol and, a few species can use acetate and the methylamines (i.e. mono-, di-, and trimethylamine) additionally; and that they are found in very reduced environments where there exist anaerobic conditions and the absence of light, NO_3^-, S^o, and SO_4^{2-} (55). These organisms can be mesophilic, thermophilic or psychrophilic. The pH optimum for growth of methanogens is near neutrality.

Technology

Empirically, biogas production appears to be a very simple operation consisting of mixing waste material with water in a digester-like tank and allowing the mixture to ferment anaerobically for some period of time, during which a variable amount of gas can be produced. This misunderstanding of the process has been the cause of failure of many pilot units, resulting in a slowdown in the integration of the biogas system in developing countries (56). Biogas production must be carefully controlled before it can be turned into a convenient and profitable operation. Much research is taking place in regard to mastering the process (56). Actually, the technology of biogas is centered around two aspects: 1) engineering designs of appropriate digesters, and 2) identification and control of factors that seem to affect the efficiency of the process, as evaluated by both the rate of gas

production and the yield of gas produced (56).

Digester. The digester is the container in which the fermentation takes place. Typically, a digester has an inlet for introduction of materials to be digested, one outlet for exit of gas and another one for liquid (slurry) effluent, and optional gas storage, mixing and heating devices. Various types of digesters exist from the most rudimentary Chinese and Indian models for rural applications to the most sophisticated industrial continuously stirred tank reactor (CSTR) mode. Also, new designs and materials of construction to build inexpensive and reliable digesters are being investigated (57). The purpose of the anaerobic digestion dictates which factors need to be given particular attention in digester design. The process can be either for stabilization of waste materials, for public sanitation, or for production of energy. When the latter is the main interest, it is particularly important to optimize the operation (58). The principal factors that can be manipulated in order to maximize the production of gas are: 1) biodegradability of the materials, measured in one of two ways: chemical oxygen demand (COD) or volatile solids (VS) destruction; 2) concentration of the feedstock as per cent total solids (TS). The more concentrated the solids, the smaller the digester and the lower the cost of the system; 3) hydraulic retention time (HRT), which is the theoretical time that a particle or volume

of liquid added to a digester would remain in the digester; and 4) kinetic constants for methane production. The kinetic constants vary with temperature and represent an index of digester performance (58). Operational temperature is a variable that also influences the design of a digester. In developed countries, the trend has been toward thermophilic digestion, and, automatic heating units have to be installed in the system in order to maintain a constant high temperature. That represents significant additional costs. In developing countries, mesophilic digestion (ambient temperature) is the mode and only the first three factors mentioned earlier can be varied to optimize the process (58). No additional costs are involved in the ambient digestion but, the production of biogas declines significantly during nighttime when the temperature drops. For design purposes, gas production rates per unit volume are often used. These rates are temperature dependent. A kinetic model which describes the mathematical relationships among the four factors presented above has been developed (59). It serves to predict the volumetric methane production and to determine the ultimate methane yields. Since the latter are hard to determine in the field, the concept of volatile solids available for conversion is used and the volumetric efficiency (m^3 gas/m^3 of digester per day) is derived instead (60).

The mathematical relationship mentioned before is expressed by the following equation:

$$V = (B_o S_o / HRT) (1 - k / (HRT_{\mu_m} - 1 + k))$$

where V is the volumetric methane rate in m^3/m^3 of digester

B_o is the volumetric methane yield in m^3 CH_4/kg VS

S_o is the influent volatile solids concentration in kg/m^3

HRT is the hydraulic retention time in days

μ_m is the maximum specific growth rate of microorganisms in $days^{-1}$

k is a dimensionless kinetic parameter.

Relations between k and S_o, and also expressions of μ_m as function of temperature (T) have been deduced (61, 62).

Anaerobic Digestion of Banana Peels

Few studies on anaerobic digestion of banana peels have been made during the last six years in developing countries where the cultivation of banana is an economic priority. International projects include from the Philippines: Utilization of Food Waste Materials for Energy, Food and/or Animal Feeds Production. Biogas from Dried Banana Peelings (34); from Costa Rica: Measurements of the Production Potential of Methane from Ripe Banana Peels (Musa spp.) (35); and from Colombia: Biogas Production from Banana Residues (63).

Dried ripe and unripe banana peelings have been investigated as a possible substrate for biogas production (34). The general procedure consisted in sun-drying the banana peelings for 7 - 14 days until rendered thoroughly dry and ground fine in a cofffee grinder. Experiments were carried out using 3 one-liter capacity bottles placed side by side and connected to each other by means of glass and rubber tubings. Each bottle contained 10% total solids in the slurry and 1% inoculum (methane starter) was added before sealing the bottles anaerobically. The evolved gases passed through the water contained in a 500-ml capacity bottle and then to a miniature gas holder. The volume of gas produced was measured and flammability tests were monitored daily. Digestion was performed at ambient temperature for a period of 20 days. It was found that the unripe samples produced 10% more methane gas than the ripe peels. Unripe banana peelings gave a cumulative gas production of 5,000 cc/3 liters of slurry (0.019 m^3 CH_4/kg VS), while ripe samples produced 4,500 cc/3 liters of slurry (0.017 m^3 CH_4/kg VS). Nevertheless, it was pointed out in that paper that since preliminary laboratory experiments conducted utilizing fresh banana peelings showed very slow degradation even after one month of anaerobic fermentation resulting in low gas production, hence fresh banana peelings were not used in this study.

In Biogas Production from Banana Residues (63), green mature banana pulp and peels were used separately. Moisture, nitrogen content and total carbon content were analyzed previously, revealing the need to add nitrogen in order to adjust the C/N ratio of both the pulp and the peels to a value of about 30, which is considered to be optimum for biogas production. The nitrogen was added in the form of urea. The pulp and peels were ground separately, and, for the digestion of these materials, 7.5 kg of pulp and 5.5 kg of peels were placed in separate digesters submerged in water, which was added in the proportion of 1:1 (wt/wt), with 1 kg of cow manure to seed the necessary bacteria. Required amounts of urea were added to obtain the adequate C/N ratio. During the digestion, which lasted 21 days, the temperature was maintained constant at 25°C, acidic conditions prevailed with pH varying from 3.7 initially to a final value of 5.

In this experiment, no biogas was produced by the green mature peels even after four months of incubation. Two explanations were given for that. The high level of fiber in the peels made it difficult to degrade, and the acidity of the peel would have inhibited the cellulolytic or fermentative bacteria so that no fermentation could have taken place and hence no production of biogas. The pulp produced 0.0483 m^3 biogas/kg VS, which is equivalent

to 0.034 m^3 CH_4/kg VS. Although this was a low yield, the obtention of biogas with more than 50% methane under the prevailing acidic condition was considered an important result. In the literature it had been reported that no biogas production was obtained at pH value below 6.

The methane production potential of ripe banana peel was studied (35). Two sets of experiments were performed, one at laboratory level according to the method described by W. F. Owen et al. (64) using 250-ml capacity bottles and another one at pilot plant scale using a digester of 17.5 liters of capacity. Fresh and partially dried banana peels were used in preliminary studies which showed very low gas production for the dried material. Therefore, dried banana peels were not used in the subsequent experiments. Operational conditions for the batch system (laboratory scale) were 35°C, 20% inoculum, C/N ratio of 48 as determined for the fresh peel (not by direct analysis but by difference), and fermentation duration time of 30 days. For the continuous system (pilot scale) the duration was 40 days and per cent total solids was 8.5.

It was found that similar results were obtained for both the batch system and the continuous system experiments. The mean value for the yield of biogas, in both cases, was 0.163 m^3 biogas/kg VS (or 0.114 m^3 CH_4/kg VS). The composition of the biogas was 70% methane and 30% carbon dioxide. Compared to biogas yield of cow manure

(0.30 m^3 biogas/kg TS), this yield for banana peel was considered low. Possible explanations were that the C/N ratio might have been too large; that the peel might have been contaminated with chemical used in the field, or, that the tannins and some metals in the peel could have affected the digestion.

There is scanty information available on the saccharification of fruit wastes, and the practice of pretreating the waste feedstock with saccharifying agents for anaerobic digestion is not reported. In India, the procedure has been used to maximize the yields of alcohol obtained from dried powdered banana peels fermented with Saccharomyces cerevisiae var. ellipsoideus (65). The peels were found to contain 31, 205, 225 mg/g peels of reducing sugars, starch and cellulose, respectively. Therefore, saccharification of cellulose was considered an essential pretreatment for alcoholic fermentation. Saccharifying agents used in that study were sulfuric acid (H_2SO_4), acid and steam, or enzyme (cellulase). The cellulase was produced, in the medium of Shewale and Sadana, by the fungus Trichoderma reesei QM 9414, obtained from the US Army Natick Research Laboratories. The effect of acid hydrolysis as pretreatment was studied utilizing 1 g of peels with 8 ml of H_2SO_4 at concentrations ranging from 2.5 to 15%, and pressurizing the solutions at 10 or 15 psi for different periods of time up to 60 min. For the enzyme-pretreatment, 50 ml of culture

filtrate from T. reesei and 50 ml of citrate buffer (0.1M, pH 4.8) were added to 5 g of pulverized peels and incubated at 50°C for 24, 48 or 72 hours. The parameter used for evaluating the performance of the different pretreatments was the saccharification value which was expressed on a percentage basis (% of cellulose for cellulase, and % of carbohydrate for the other saccharifying agents). It was found that the maximum saccharification value of 87.8% was achieved by treating the banana peels with cellulase for 72 hours. This saccharification value corresponded to a production of 224 mg/g peels of reducing sugars. The second best saccharification value was obtained for the treatment of 2.5% H_2SO_4 at 15 psi for 15 min. This value was equal to 59.9%, but, it represented the highest production of reducing sugars estimated at 307 mg/g peels. Consequently, these two best pretreatment conditions for the saccharification of banana peels were chosen for the ethanol fermentation. Aliquots (150 ml) of each acid-, and enzyme-pretreated substrate were sterilized and then inoculated with 3% (v/v) yeast inoculum at 30°C. Alcohol yield, expressed as ml of alcohol per 100 g of reducing sugars, was determined after 24 and 48 hours of fermentation. The corresponding alcohol yields for the cellulase pretreatment were 61 and 60, respectively. These values were higher than the alcohol yields obtained after the acid pretreatment (45 and 44). Finally, it was concluded that from the results on the saccharification of

banana peels by cellulase and the efficiency of S. cerevisae var. ellipsoideus in converting hydrolyzed banana peels to ethanol, a yield of 150 ml of alcohol per kg of banana peels can be obtained. This is three times the yield of 43 g of ethanol per kg of apple powder recorded by Hang, Y. D..

In summary, the previous works on bioconversion of banana peels available in the literature showed that green mature banana peel was not digestible (63); that dried unripe banana peel produced more biogas than dried ripe banana peel and that fresh ripe banana peel was not digestible (34); and that fresh partially dried banana peel was poorly digestible, and low production of biogas (0.15 m^3 of biogas/kg TS) was obtained with ripe banana peel (35).

CHAPTER III
OBJECTIVES AND JUSTIFICATIONS

The objectives of the present research were 1) to determine the technical feasibility of anaerobic digestion of fresh ripe banana peels, and 2) to determine the effects of prior saccharification of banana peels by acid, base and/or enzyme upon the improvement of the yield of the methane generated during the fermentation.

The justifications for this work consisted in that: a) anaerobic digestion of fresh ripe banana peels had not been reported and we believed that, due to its chemical composition and other characteristics, fresh ripe banana peels could be made digestible; b) sun-drying which is performed in tropical countries is not commonly available in many industrialized countries and therefore, the drying process would require another form of energy and associated additional cost. Also, the sun-drying process in a large-scale operation would require a great surface area. The concomitant infection of the zone and the material with insects and other contaminating vectors would represent a public health safety problem. It has also been suggested that fresh wastes should be utilized whenever possible since, the longer they are exposed to environmental degradation, the smaller will be the

biodegradable fraction available for methane production; c) contrary to some discouraging reports (35, 63) about the anaerobic digestion of that substrate, we have obtained through a preliminary biochemical methane potential (BMP) assay the first clear indications that fresh ripe banana peels can be used to produce methane, and d) we believed that as H. K. Tewari in his study of production of ethanol from banana peels (65) did improve the alcohol yield by pretreating the peels with saccharifying agents (i.e. enzyme or acid), a similar improvement might also be obtained in conversion to methane. The observed improvement may have been due to the swelling action on the structure of the biomass and/or the rapid fermentation of sugars released during saccharification.

CHAPTER IV
MATERIALS AND METHODS

Development of the Biochemical Methane Potential Assay

The biochemical methane potential (BMP) assay involves (1) preparation of culture medium, inoculum, and substrate, (2) development of the BMP assay per se, and (3) determination of performance parameters such as pH, biogas measurement, biogas composition, and methane yield and production rate constant.

Medium

The culture medium was prepared according to the method described by W. F. Owen et al. (64).

Inoculum

The inoculum was obtained from a 5-liter working volume mesophilic (35°C), continuously mixed digester using primary municipal sludge received from Gainesville City Municipal Sewage Plant. The digester had been fed daily for 3 years. Owen's medium was added to the sludge on a 20% v/v (medium/sludge) basis. Methane was obtained from this digester in the range of 60 - 65% of theoretical yield from volatile solids.

Substrate

Ripe bananas (grade 5) were purchased from a local food store. For use as substrate for BMP studies, the banana peels were first submitted to two separate physical treatments. Required chemical analyses were then performed on the modified samples, which occasionally were hydrolyzed either with enzymes or with acids.

Homogenization. Fresh banana peels were sliced into small pieces, macerated with a spatula in a 25-ml plastic centrifuge tube, and passed through a Polytron (Brinkmam Model PT 10/35) that converted the fibrous material into a brownish homogenate. The homogenate sample of fresh banana peels was placed in a Ziploc (Dow, 7 in x 5¼ in x 2.7 MILS) plastic bag that was labelled for identification of the sample, and stored frozen at - 20°C.

Drying. Cut banana peels were arranged in oven pans, previously covered with aluminum foil, to form a uniform layer, and dried at 150°C for 6 hours in a kitchen oven. The dark, thoroughly dried, and friable pieces were collected, placed into a Mini-blend container (Osterizer 4.0 oz), and then ground in a Osterizer kitchen blender for 30 sec or until a fine powder was obtained. The pulverized dried peel was placed into a similar Ziploc plastic bag, labelled for identification of the sample, and preserved frozen at - 20°C.

The chemical analyses consisted of determining moisture content, total solids (TS), volatile solids (VS), ash content, nitrogen, and chemical oxygen demand (COD). The COD was determined according to the Hack method (66). All the other determinations were done following the procedures outlined in "Standard Methods for the Examination of Water and Wastewater" (67).

Enzymatic hydrolysis. Two enzyme solutions were prepared. A solution of cellulase was made by dissolving 4 g of Trichoderma (T. reesei QM 9414) cellulase enzyme in 400 ml of deionized water (pH 4.14). Another solution of pectinase was prepared by diluting 10 ml of a commercial preparation (NOVO Pectinex Ultra SP) to 500 ml with deionized water (pH 4.91). From either solution, 10 ml were taken and added to the fermentors (Corning No. 1460, 250-ml serum bottles) containing proper amount of substrate (i.e. dried or fresh banana peel, or cellulose). The fermentors were shaken for a short period of time and the pH of the resulting suspension, when required, was adjusted to a value of 5. The fermentors were sealed and left at room temperature for a period of 24 hours. For the BMP analysis of the enzymatically-pretreated samples, the pH of fermentors was adjusted to neutrality with aliquots of 0.5 N NaOH solution. Thereafter, the volume in each fermentor was brought (under anaerobic conditions) to 100 ml by adding 70 ml either of deionized water or of Owen's

medium plus 20 ml of inoculum. For each case, enzyme blank fermentors (i.e. without banana peel or cellulose) were also prepared.

Acid hydrolysis. Fermentors containing a suspension either of fresh or of dried banana peel sample equivalent to 0.2 g VS in 10 ml either of 0.5 N NaOH or of 0.5 N H_2SO_4 were autoclaved at 10 psi for 30 min. After cooling at room temperature, the pH was adjusted to neutrality. The volume of fermentors was then brought to 100 ml in the same fashion as for the enzymic treatment described above.

The BMP procedure

Clean and dried Corning No. 1460, 250 ml (264 $\pm$1 ml actual volume with Bittner size No. 16 serum cap in place) served as fermentors. A flow-diagram of the BMP process is outlined in Figure IV-1. Three major stages are identified.

Stage 1. The weight of empty, labelled fermentors was recorded. Amount of sample calculated to contain 0.2 g of VS was placed in corresponding fermentors which were gassed with a gas mixture composed of CO_2 (39.2%) and N_2 (60.8%) at a flow rate of approximately 0.5 liter per minute for 25 minutes. The gas mixture had been passed through a hot copper column which previously was purged with hydrogen gas. To prepare a 20% inoculum on a v/v basis, 3,200 ml of Owen's medium and 800 ml of inoculum were mixed thoroughly at 35°C by a magnetic stirrer in a 4-liter reservoir bottle, with sparging with the CO_2:N_2 mixture under anaerobic conditions.

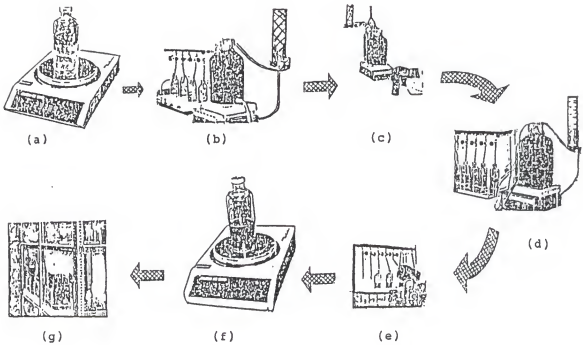

Figure IV-1. Flow Diagram of BMP Process for Banana Peels.

Stage 1: (a) weighing of empty fermentors, and (b) flushing of substrate with CO_2:N_2 mixture. Stage 2: (c) inoculation with 100 ml seeded medium, (d) flushing, and (e) capping of fermentors. Stage 3: (f) weighing of inoculated fermentors, and (g) incubation of fermentors at 35°C.

Triplicate fermentors for each sample and also for the control or seed-blank (with no organic substrate added) were prepared.

Stage 2. A volume of about 100 ml of seeded medium was transferred anaerobically to each fermentor, which then was gassed for 20 minutes. At the end of the gassing period, a rubber serum cap was inserted while removing the gas flushing tube from the fermentor.

Stage 3. Finally, after securing the rubber cap with a 20-mm tear-off aluminum seal (Wheaton Scientific) and recording the weight of the fermentors, those were placed in a rack and incubated at 35°C in an incubator (Nor-Lake Scientific Environmental Room S/N 87301778) for a total of 30 days. At the end of the incubation period, the constituents from the three replicates were mixed together and analyzed for remaining volatile solids and for pH.

Determination of parameters

Gas measurement. Periodically during incubation, gas-volume sampling and removal were performed. Once the fermentors were removed from the incubator, they were placed in a water bath (Fisher Scientific Versa-Bath S, Model 224) at 35°C for 45 min. After equilibration, the volume of biogas produced was determined according to Owen's syringe procedure (64).

Gas composition. Methane and carbon dioxide concentrations were determined in a Gow Mac-580 gas cnromatograph (GC) equipped with a thermal conductivity detector. A stainless steel column (8 ft by 1/4 inchs ID) packed with 80/100 Porapak Q was used. Helium was used as the carrier gas at a flow rate of 60 ml min^{-1}. The temperatures of the column, injector and detector were 60, 90 and 90°C, respectively. A certified gas standard composed of 22.29% N_2, 3.08% O_2, 14.98% CO_2 and 59.65% CH_4 was used for calibration of methane and carbon dioxide.

Methane yield. Methane yield B (ml CH_4/g VS) was calculated by subtracting a proportional amount of the methane produced by the controls (average production from three controls) from the methane production of each treatment fermentor, then dividing the difference by the amount of VS in the sample (0.2 g). An example of how the net cumulative methane yield is calculated is given in Table IV-1. By plotting the net cumulative methane yields against the corresponding time, a curve of sigmoidal shape, characteristic for methane production, is obtained (Figure IV-2). Ultimate methane yield B_o (m^3 CH_4/kg VS) and methane production rate k (per day) were calculated using least squares fit of the data (PRIMOS version of SAS release 5.03, 1985) to the following equation:

$$B = B_o(1 - e^{-kt})$$

where t is the time in days (68).

Table IV-1. Determination of Methane Yield From Cumulative Volumes of Methane Gas Produced During the Digestion of the Sample.

Illustration

	Time (days)		
	2	6	10
a) Gas evolved (ml)	64	20	18
b) Head space volume (ml)	170	170	170
c) % CH_4 determined by GC	11.6	13.4	19.5
(c) x (a) = CH_4 (ml) in gas evolved	7.42	2.68	3.51
(c) x (b) = CH_4 (ml) in head space	19.72	22.78	33.15
Subtotal CH_4 (ml)	27.14	25.46	36.66
Total CH_4 (ml) = Subtotal plus CH_4 evolved during previous days	27.14	32.88	46.76

Net Total = Total (sample) - Total (control)

Methane Yield = Net Total CH_4 (mls)/g VS

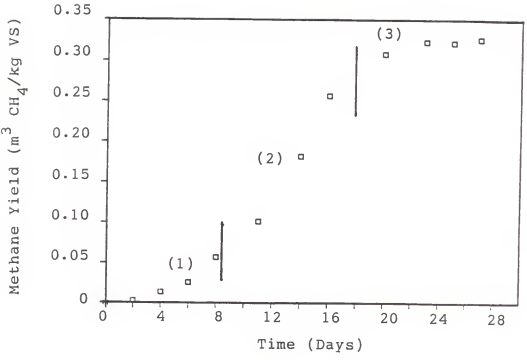

Figure IV-2. Methane Production Curve.

Segment (1) of the curve represents the lag phase which is the adaptation period of the microorganisms to the particular environment. Segment (2) is the log phase where the production of methane is faster. Segment (3) is the stabilization or equilibrium phase where the ultimate methane yield is reached.

CHAPTER V
EXPERIMENTS AND RESULTS

A preliminary experiment explored the bioconversion to methane of the components of ripe bananas, the pulp and the peels both separately as well as combined (whole banana). No previous reports existed of the bioconversion of either whole banana or the fresh peel. In all three cases, the natural substrates were converted into a puree by blending a 1:1 suspension (on a weight basis) in an Osterizer blender, and the BMPs were conducted following the general process described in Chapter IV. After the 20 days duration of this preliminary experiment, positive indications of the feasibility of producing methane from ripe fresh banana peels were obtained contrary to literature reports (34 - 35). The methane yields for the banana pulp, whole fruit, and the peels were equal to 0.31, 0.29 and 0.17 m^3 CH_4/kg VS, respectively.

Since fresh ripe banana peels thus were shown to be anaerobically digestible, these were selected as the substrate for further investigation. Another preliminary experiment had as its goal the identification of experimental conditions for improved methane yield from ripe fresh banana peels. In order to achieve that goal, the following

strategic plan was designed:

1.- Complete BMP assays on both fresh and dried banana peels either as natural or as pre-saccharified samples,

2.- Determine the effect, if any, of omitting the nutrients provided in the Owen's culture medium upon the efficiency of the digestion, since it was predicted from the mineral composition of the peel that the latter could supply many of the microbial nutrient needs otherwise supplied by the complex Owen's medium.

In this experiment, homogenized fresh and pulverized thoroughly dried banana peels served as substrates. One set of samples were digested in the Owen's medium, and another set of samples were digested omitting the Owen's medium and substituting a corresponding volume with water. A similar experimental design was followed for digestion of the pre-saccharified substrates. Results for this experiment are presented in Table V-1, and these show that fresh and dried banana peels have the same methane yield potential, that the addition of the Owen's medium has no positive effects upon the performance of the digestion, and that cellulase pretreatment can enhance methane yield. Table V-1 also shows that fresh banana peels produced the same methane yield (0.20 m^3 CH_4/kg VS) in either case. This result for the fresh peel was expected and suggested that the peel effectively satisfied the mineral needs of the microbial

Table V.1. Methane Yield of Pre-saccharified Fresh and Dried Banana Peels After Digestion in Presence or Absence of Owen's Medium.

	Owen's Medium			
	+		-	
	Fresh	Dried	Fresh	Dried
Pretreatment	(m^3 CH_4/kg VS)			
Control	0.20	0.20	0.20	0.30
Cellulase	0.28	0.26	0.34	0.34
H_2SO_4	0.12	0.14	0.19	0.19
NaOH	0.12	0.10	0.15	0.19

agents responsible for the methanogenesis. For the dried peel, a maximum methane yield of 0.30 m^3 CH_4/kg VS was obtained without addition of Owen's medium compared to an ultimate methane yield of 0.20 m^3 CH_4/kg VS when the Owen's medium was added. Thus there was an apparent depression by about 33% by addition of exogenous nutrients. Such an effect on the methane yield from the dried peel was unexpected and suggested an unlikely inhibitory effect by the minerals of the Owen's medium. It is difficult to explain this behavior of the dried peels, since it might be caused by one or by various components of the complex Owen's medium. It was judged, thus, necessary to duplicate the experiment by providing the medium "without nutrients" with the same buffer capacity and a similar reducing environment as those in the Owen's medium. Table V-2 demonstrates that with both modifications no differences were seen between results of BMPs with or without Owen's medium for fresh and dried banana peels, which produced identical methane yields. Pure cellulose (Avicel), which was used mainly as a means to monitor the quality and availability of the cellulolytic enzymes in the inoculum, was digested under the same conditions and showed virtually no significant differences in the methane yield with or without Owen's medium. These results suggest that the ultimate methane yield was limited by the level of the redox potential of the BMP incubation milieu and,

Table V-2. Methane Yield of Fresh and Dried Banana Peels, and of Cellulose Digested in Presence or Absence of Owen's Medium With Both Milieus Having Same Level of Buffer and Equivalent Levels of Reducing Agent.

	Owen's Medium	
	+	-
Sample	(m^3 CH_4/kg VS)	
Fresh Peels	[a] 0.22 ± 0.10	[a] 0.20 ± 0.06
Dried Peels	[a] 0.22 ± 0.11	[a] 0.21 ± 0.08
Cellulose	[a] 0.34 ± 0.04	[a] 0.32 ± 0.05

[a] No significant difference; $t < 2.776$ at $\alpha = 0.05$.

combined with the results of the preceding experiment, they offer the insight that a certain optimum redox level probably exists for best digestion performance.

Anaerobic Digestion of Enzyme-pretreated Banana Peels

It was found previously that cellulase-pretreated samples digested either with or without Owen's medium produced higher methane yields than the corresponding controls (Table V-1). Since banana peels have a relatively high content of pectins, it was considered that pretreatment of the peels with pectinase could produce substrates more easily available to the methanogenesis because the commercial pectinase preparation contains cellulase and other activities. Therefore, digestions of cellulase-, and pectinase-pretreated samples were compared. The BMPs were conducted under standard procedures (i.e. according to the Owen's method). The methane yield from the enzyme-pretreated samples (Table V-3) shows that enzyme-pretreatment of the peels had no influence on the ultimate methane yield after the contribution of the enzyme was subtracted. The rate of methane production (Table V-4), nevertheless, shows significant differences. It was observed that with banana peels, pretreatment with pectinase was more effective than that with cellulase since the methane production rate was about 100% more than the control values for fresh as well as for

Table V-3. Methane Yield of Enzyme-pretreated Cellulose, Fresh and Dried Banana Peels.

Sample	Pretreatment		
	Cellulase	Pectinase	Control
	(m^3 CH_4/kg VS)		
Fresh Peels	[a] 0.24 ± 0.07	[a] 0.25 ± 0.11	[a] 0.22 ± 0.10
Dried Peels	[a] 0.22 ± 0.02	[a] 0.24 ± 0.03	[a] 0.22 ± 0.11
Cellulose	[a] 0.34 ± 0.11	[a] 0.35 ± 0.09	[a] 0.34 ± 0.04

[a] No significant difference; $t < 2.776$ at $\alpha = 0.05$.

Table V-4. Methane Production Rate Constant (k) for Enzyme-pretreated Cellulose, Fresh and Dried Banana Peels.

Sample	Pretreatment		
	Cellulase	Pectinase	Control
	($Days^{-1}$)		
Fresh Peels	[a]0.13 ± 0.01	[b]0.27 ± 0.03	[a]0.12 ± 0.01
Dried Peels	[a]0.14 ± 0.02	[b]0.30 ± 0.04	[a]0.15 ± 0.02
Cellulose	[a]0.17 ± 0.02	[a]0.14 ± 0.03	[a]0.10 ± 0.02

[a] No significant difference; $t < 2.776$ at $\alpha = 0.05$.

[b] Significant difference; $t > 2.776$ at $\alpha = 0.05$.

dried banana peels. Cellulase pretreatment showed no significant differences from the control values. Comparing Figures V-1 (control), V-2 (cellulase pretreatment) and V-3 (pectinase pretreatment), it can be seen that, in all three cases, the same ultimate methane yield was obtained for both fresh and dried banana peels. However, with pectinase pretreatment the ultimate methane yield was reached in the minimum time period of 8 days as compared to 13 days for cellulase pretreatment and 15 days for non pretreated peels. The above observation reveals some differences in the methane production rate. HPLC analysis of the enzymatically pretreated banana peels indicated differences after pretreatment in the pectin content. Figure V-4 shows that much more pectin breakdown product (galacturonic acid) was yielded by pectinase-pretreated banana peels than by cellulase-pretreated samples. That could explain the different range of improvement which is observed for the rate of methane production (Table V-4).

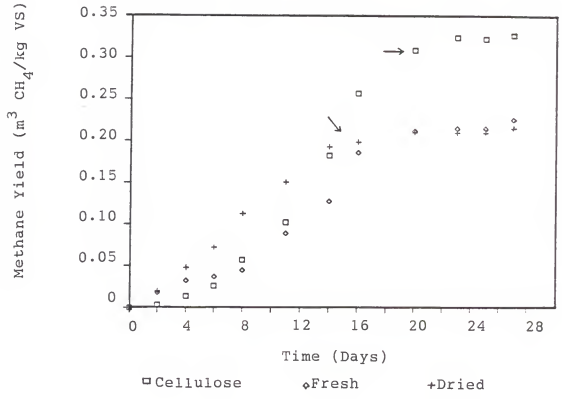

Figure V-1. Methane Production From Cellulose, and Natural Fresh And Dried Banana Peels (Controls).

(⟶) indicates when ultimate methane yield is reached.

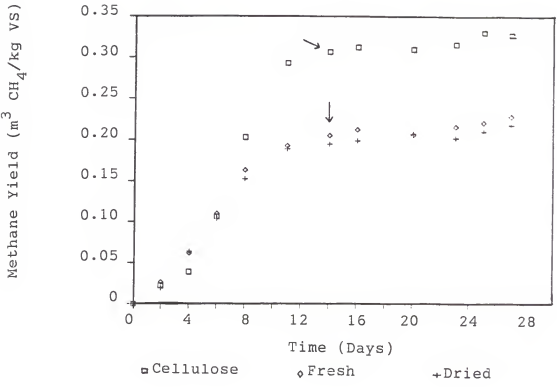

Figure V-2. Methane Production From Cellulase-pretreated Cellulose, Fresh and Dried Banana Peels.

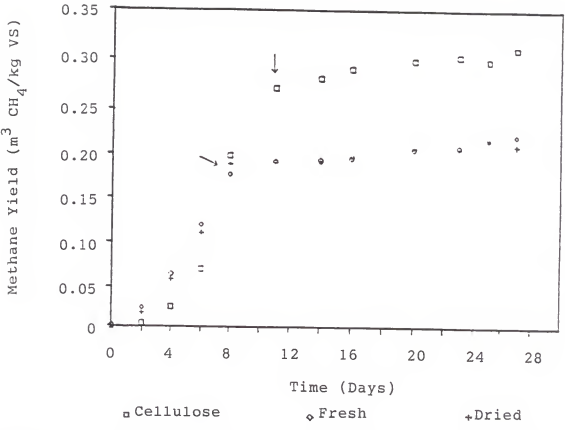

Figure V-3. Methane Production From Pectinase-pretreated Cellulose, Fresh and Dried Banana Peels.

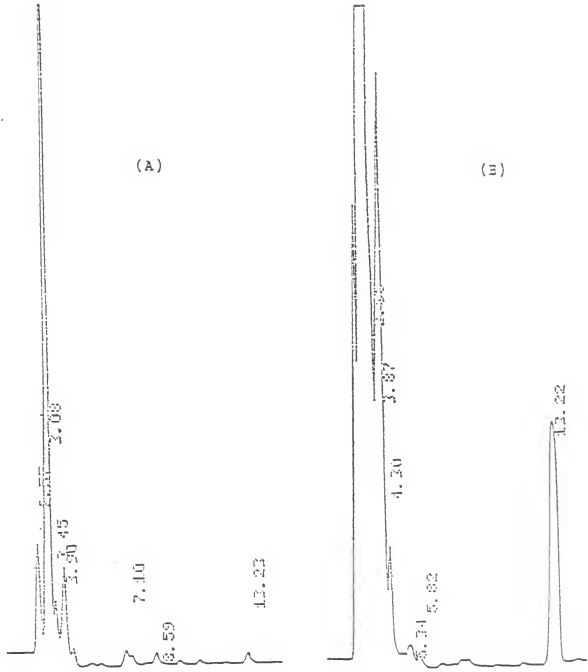

Figure V-4. Chromatographs of Soluble Sugars From Cellulase-, and Pectinase-pretreated Banana Peels After 24-hr Incubation.

(A) = Cellulase-pretreated Samples
(B) = Pectinase-pretreated Samples.

CHAPTER VI
DISCUSSION

From an investigation in the Philippines (34), ground sun-dried ripe banana peels were found to be a feasible substrate for anaerobic digestion. Using as a fermentor a one-liter capacity bottle containing 10% solids in the slurry and 1% inoculum, an ultimate methane yield of 0.02 m^3 CH_4/kg VS was obtained after 20 day-duration of this study. No mineral salts were added, pH was adjusted to 7 and no reducing agents were used. Assuming that a similar methane yield would have been obtained if the BMP had been conducted in a smaller volume (e.g. 100 ml), we can thus compare this result to the one we have obtained for the anaerobic digestion of dried ripe banana peels digested without using Owen's medium (Table V-1). Under the conditions of our experiment, an ultimate methane yield of 0.30 m^3 CH_4/kg VS was obtained. This large difference in the performance of the digestions (0.30 vs. 0.02 m^3 CH_4 per kg VS) may be due to the degree of ripeness of the peel, to the strenght of the inoculum or to the drying process used in the other study. The sun-drying process for a period of 14 days prior to the digestion might have affected the concentration of the degradable portion in the banana peel. It is possible that loss of degradable solids

might occur due to consumption by insects attracted to the peels and/or by natural microbial degradation.

Quesada Meseguer (35), in Costa Rica, using Owen's procedure as we did to examine the methane potential of ripe banana peels, reported an ultimate methane yield of 0.11 m^3 CH_4/kg VS compared to 0.22 m^3 CH_4/kg VS observed in our experiment. The peels used in both studies contained the same C/N ratio of 48 and also the same grade of ripeness of 5. The difference between results can be attributed to the physical pretreatment to which the banana peels were subjected. The homogenization of the peels using the polytron which converts fibrous materials such as banana peels into a more homogeneous, pasty substance which forms a uniform slurry offering a larger total particle surface area thus permitting better substrate-microorganism contact, which is ultimately translated into improved digestion.

Contrary to studies elsewhere which are cited above, we have been able to show that either fresh or dried banana peels have the same methane yield potential. Oven-drying, if it can be made cost efficient, could be a way to preserve the peels for methane production without loss of the biodegradable fraction. Calculated COD values for the fresh (0.8% suspension) and dried peels (0.3% suspension) were 900 and 1,000 mg/L, respectively.

For the first time, in a single study, three specific factors believed to affect conversion efficiency have been

investigated: (1) enzyme-pretreatment of the banana peels, (2) omission of exogenous mineral salts, and (3) additon of reducing agent alone. Enzyme-pretreatment of the peels with cellulase or pectinase was found to have no effect upon the ultimate methane yield when correction was made for the contribution of each particular enzyme to methane conversion. Only the pectinase-pretreatment improved the rate of methane production with increases in rate being of 100% for dried as well as for fresh banana peels. This significant increase in the rate reflects the fact that the methanogenic system did not need to produce the enzymes necessary for optimal degradation of the complex substrate. The peel, when broken down by the pectinase sustains a faster rate of conversion to methane and attains the ultimate methane yield (stabilization zone) in a shorter period of time. This behavior is shown clearly in the reduction of the lag phase (Figure V-3 vs. Figure V-1) which represents the phase of adaptation of the microorganisms to the particular milieu or substrate. This finding is important for digester design since the reduction of the lag phase should result in shorter residence times and consequently smaller digesters and most importantly, reduced capital cost.

For the anaerobic digestion of banana peels, pectinase was found to be more effective than cellulase or other hydrolytic pretreatments. Nevertheless, it can be

assumed that for each enzyme the optimum concentration and incubation parameters are yet to be determined for best conversion efficiency. Also, it would be interesting to investigate the effect of several combinations of these two enzymes at different levels upon the performance of the BMP of the banana peels. Because the cost of commercial enzymes may render them uneconomical for use in pretreatment of biomass destined to biogas production, a useful long-term goal could be the development of digester compatible anaerobes which would produce, in situ, higher levels of the necessary enzymes for the bioconversion process.

We have also shown that banana peels either as natural or as modified samples can support good methane yield without mineral supplementation. For samples of unmodified dried or fresh banana peels, final pH values after anaerobic digestion in the presence of buffer are not significantly different from final pH values measured when no buffer had been added (7.2 ± 0.03 vs. 7.4 ± 0.09). This result may indicate a intrinsic buffering capacity of the ripe banana peel in the range of 7.3 ± 0.1, suitable for anaerobic digestion. The question of the effect of the level of the reducing agent (or redox potential) on the digestion of a particular substrate in the slurry has also been raised.

With a methane yield of 0.45 m^3 CH_4/kg VS added being considered as 100% VS conversion (69), the methane yield obtained in our study for the anaerobic digestion of ripe

banana peels, 0.25 m^3 CH_4/kg VS added, corresponds to a conversion of VS of 51%. Compared to an efficiency of conversion of 40% for the anaerobic digestion of most vegetables (71), the result for banana peels is satisfactory. However, we would like to suggest some procedures that might improve the conversion efficiency of the banana peels. These include: (1) the supplementation of the peels with an inexpensive nitrogen source such as urea in order to standardize the C/N ratio in the range of 14 - 23 (50); (2) the determination of the optimum grade of ripeness of the peel for bioconversion where the concentration of active tannins in the peel would be at a considerably low level (Table II-2). Tannins were found able to inhibit the metabolic process of various anaerobes (70); or (3) washing of the peel with water in order to eliminate residual fungicides, pesticides and/or herbicides. The latter were reported to be highly toxic to the anaerobic digestion process when they were used at concentration of at least 5 mg/L (71).

In summary, we can state that the principal objective of our study has been achieved. The technical feasibility of fresh ripe banana peels to produce methane gas was established. Beyond that, innovative approaches for carrying out biochemical methane potential assay were also introduced.

CHAPTER VII
CONCLUSION

To rely on depleted or diminishing natural resources for satisfying disproportionately large and increasing needs of mankind seems to be an unreachable objective. This dilemna is a reality of the times in which we live. It appears, thus, urgent to search for ways to improve the prevailing conditions. In such times of crisis, any effort to contribute to solutions is commendable. An earlier contribution of the author was in the production of inexpensive animal protein by developing a low-cost, protein-rich animal feed using whole banana waste (21). Food shortages, in general, and cost-prohibiting access to animal protein in many less developed countries (LDC) represent a challenge to be overcome. In this present work, production of energy from renewable sources, a timely necessity, has been chosen as the domain for another contribution. Methane gas has been produced from banana peels which constitute a vast amount of digestible biomass. A firm basis has been established to pursue the conversion to methane from this substrate at maximum efficiency. However, since this work was performed at the laboratory level, no conclusive

information can be obtained from it about the bioconversion of banana peels in a larger system. It is recommended, then, that the process be scaled-up and that selection of a convenient digester be made in order to evaluate adequately the performance of this process. Also, the study of the economical feasibility of converting banana peels into energy (methane) in a rural setting is worth considering for practical purposes.

Biogas production technology, besides being a simple and inexpensive means of converting otherwise wasted materials into valuable products (such as energy in the form of methane, fertilizer and/or animal feed), also serves as powerful tool to sanitize the environment. The benefits associated with biogas technology represent an advantage over other methods (i.e. incineration, landfill etc.) for solid waste management. The integration of biogas technology into agriculture, the principal basis of third world economies, is to be highly recommended since this policy will favor the stabilization of the economy in those countries.

REFERENCES

1. Gardel, A. (1979) Energy; Economy and Perspective. Pergamon Press, New York.

2. El-Halwagi, M.M. (1986) Biogas Technology, Transfer and Diffusion. Elsevier Science Publishing Co., Inc., New York.

3. Hobson, P.N., Bousfield, S., and Summers, R. (1983) Methane Productionfrom Agricultural and Domestic Wastes. Halsted Press Div., Wiley, New York.

4. Anonymous (1987) Bananas. International Fruit World 41: (3) 127-151.

5. Stover, R.H., and Simmonds, N.W. (1987) Bananas. John Wiley and Sons, Inc., New York.

6. Chaw, H.T. (1983) Handbook of Tropical Foods. Marcel Dekker, Inc., New York.

7. Shaw, N. (1980) Tropical and Subtropical Fruits: Composition, Properties and Uses. Avi Publishing, Inc., Westport, Connecticut.

8. Firman, I.D. (1972) Susceptibility of Banana Cultivars to Fungus Leaf Diseases in Fiji. Trop. Agric. Trin. 49: 189-196.

9. Seelig, R.A. (1969) Bananas. Fruit and Vegetable Facts and Pointers. United Fresh Fruit and Vegetable Assoc., Washington, DC.

10. USDA (US Dept Agric.) (1963) Composition of Foods. US Govt. Print. Off., Washington, DC.

11. Gasster, M. (1963) The Banana in Geriatric and Low-calorie Diets. Geriatrics 18: 782-786.

12. Marriott, J. (1980) Bananas: Physiology and Biochemistry of Storage and Ripening for Optimum Quality. Crit. Rev. Food Sci. Nutr. 13: 41-48.

13. Apelbaum, A., Aharoni, Y., and Gorodeiski, T.N. (1977) Effects of Subatmospheric Pressure on the Ripening Processes of Banana Fruits. Trop. Agric. Trin. 54: 39-46.

14. Peacock, B.C. (1972) Role of Ethylene in the Initiation of Fruit Ripening. Queensl. J. Agric. Anim. Sci. 29: 137-145.

15. Growter, P.C. (1979) The Processing of Banana Products for Food Use. G122. Tropical Products Institute Publ., London.

16. Clavijo, H., and Maner, J.H. (1974) The Use of Waste Bananas for Swine Feed. International Center for Tropical Agriculture, CIAT, Colombia.

17. Chesnot, M. (1976) Possibilities of Using Bananas for the Feeding of Ruminants in Humid Tropical Regions. J. Agric. Univ. Puerto Rico 60: 516-525.

18. Correa, H.A., and Velez, A.S. (1982) "Use of Banana Waste in Feeding Ruminants (Musa paradisiaca)" (In Spanish). National University of Colombia, Colombia, Medellin.

19. Baldensperger, J., Le Mer, J., Hannibal, L., and Quinto, P.J. (1985) Solid-state Fermentation of Banana Wastes. Biotechnology Letters 7: (10) 743-749.

20. Sethi, R.P. (1983) Evaluation of Fermented Banana Waste Product as Protein Source in Broiler Rations. The Indian Journal of Animal Sciences 53: (9) 146-153.

21. Saint-Phard, C. (1984) Protein Enrichment of Waste Banana by Solid-state Fermentation. Thesis. National Autonomous University of Mexico. Mexico, D.F.

22. Anonymous (1982) Identification and Development of A Series of Products Using Bananas of Second Category and Banana Waste (In Spanish). University of Costa Rica, Limon, Costa Rica.

23. Anonymous (1988) Bananas. International Fruit World 43: (2) 71-90.

24. Acon, W. (1988) U.S. Demand For Avocados, Bananas and Tropical Fruits: Tobit Analysis and Projections. Thesis University of Florida.

25. Forsyth, W.G.C. (1980) Bananas and Plantains. In: Tropical and Subtropical Fruits. Nagy, S., and Shaw, P.E. (eds). AVI Publishing, Westport, Connecticut.

26. Palmer, J.K. (1971) The Biochemistry of Fruits and Their Products. Academy Press, London.

27. Griffiths, L.A. (1959) Detection and Identification of the Polyphenoloxidase System of the Banana. Nature 184: 58-59.

28. Nagatsu, I., Sudo, Y., and Nagatsu, T. (1972) Tyrosine Hydroxylation in the Banana Peel. Enzymologia 43: 25-31.

29. Goldstein, J.L., and Swain, T. (1963) Changes in Tannins in Ripening Fruits. Phytochem. 2: 271-383.

30. Simpson, B.B., and Ogorzaly, M.C. (1986) Economic Botany: Plants in Our World. McGraw-Hill, New York.

31. Phillipson, J.D., Ayres, D.C., and Baxter, H. (eds) (1988) Plant Polyphenols. Academy Press, London.

32. Singleton, V.L. (1981) Naturally Occuring Food Toxicants: Phenolic Substances of Plant Origen Common in Foods. Advances in Food Research 27: 149-220.

33. von Loesecke, H.W. (1950) Bananas: Chemistry, Physiology, Technology. Interscience Publishers, Inc., New York.

34. Silverio, C.M., Tan, B.V., Pacheco, V.G., Anglo, P.G., and Abad, E.J. (1983) Utilization of Food Waste Materials for Energy, Food and/or Animal Feeds Production. Biogas from Dried Banana Peelings. NSTA Technology Journal Jan/Mar 1983.

35. Quesada Meseguer, M.C. (1983) Measurements of the Production Potential of Methane from Ripe Banana Peels (Musa spp.) (In Spanish). Thesis. University of Costa Rica.

36. Gottreich, M. (1969) The Determination of the State of Ripeness of Bananas by Colorimetry. Trop. Agric. Trin. 46: 239-245.

37. Bressani, R., Aguirre, A., Arroyave, R., and Jarquin R. (1961) Chemical Composition of Different Banana Cultivars and Use of Banana Flour in the Feeding of Chicks. (In Spanish). Turralba 110: 127-129.

38. Wilson, R.J. (1975) The International Market for Banana Products for Food Use. G103. Tropical Products Institute Publ., London.

39. Anonymous (1980) Bandeco Banana Fruit Company. ASBANA Information. Food Technology Center (In Spanish) Limon, Costa Rica.

40. Goewert, R.R., and Maner, J.H. (1980) Banana Peel Sugars as A Source of Foodstuff for Animals or Humans. Nutr. Report 22: 207-212.

41. Clavejo, V.H., and Maner, J.H. (1974) "The Use of Banana Waste in the Nutrition of Pigs" (In Spanish) International Center for Tropical Agriculture. Series EE #6: 1-20. CIAT, Colombia.

42. Calles, A., Clavejo, V.H., Hervas, E., and Maner, J.H. (1970) Ripe Bananas (Musa) as Energy Source for Growing Finishing Pigs. J. Anim. Sci 31: 197 (Abstract).

43. Chung, S.L., and Meyers, S.P. (1979) Bioprotein From Banana Wastes. Development in Industrial Microbiology 20: 723-732.

44. Le Dividish, J., Geoffroy, I., Cenope, I., and Chenost, M. (1976) Using Waste Bananas as Animal Feed. World Animal Rev. 20: 22-30.

45. Tewari, H.K., Marwaha, S.S., Rupal, K., and Singh, L. (1985) Production of Ethyl Alcohol From Banana Peels. Journal of Research, Punjab Agricultural University 22: (4) 703-711.

46. Sudhir, K., Mishra, I.M., Sharma, M.P., and Siani, J.S. (1988) Biogasification of Banana Peeling and Ipomoea fistulosa Leaves. Khadigramodyog 34: 324-332.

47. Samir, S.S., and Zaborsky, O.R. (1981) Biomass Conversion Processes For Energy and Fuels. Plenum Press, New York.

48. Stuckey, D.C. (1986) Biogas: A Global Perspective. In: Biogas Technology, Transfer and Diffusion. Elsevier Science Publishing Co., Inc., New York.

49. Archer, D.B. (1983) The Microbiology Basis of Process Control in Methanogenic Fermentation of Soluble Wastes. Enzyme and Microbial Technology 5: 162-170.

50. Loehr, R.C. (1984) Pollution Control for Agriculture. Academic Press, Inc., New York.

51. Parker, C., and Roberts, T. (1985) Energy From Waste: An Evaluation of Conversion Technologies. Elsevier Applied Science Publishers, New York.

52. Singer, A. (1973) Methane: Fuel of the Future. Prism Press, New York.

53. Mah, R.A. (1986) Microbiology and Biochemistry of Methanogenesis: A Selected Review. In: Biogas Technology, Transfer and Diffusion. Elsevier Science Publishing Co., Inc., New York.

54. Keltjens, J.T., Hubberts, M.J., Laar Hoven, V.H., and Vogels, G.D. (1983) Structural Elements of Methanopterin, A Novel Pterin Present in Methanobacterium thermoautotrophicum. European Journal of Biochemistry 130: 537-544.

55. Stanier, R.Y., Ingraham, J.L., Wheelis, M.L., and Painter, P.R. (1986) The Microbial World 5th edn. Prentice-Hall, Englewood Cliffs, New Jersey.

56. Food and Agricultural Organization (FAO) (1984) FAO Activities in the Field of New and Renewable Sources of Energy. Second Session of the Intergovernmental Committee on the Development and Utilization of New and Renewable Sources of Energy, New York 23 April - 4 May, 1984.

57. Chen, R.C. (1983) Up-to-Date Status of Anaerobic Digestion Technology in China. Third International Symposium on Anaerobic Digestion. 1983. Boston, Massachusetts.

58. Ward, R.F. (1986) Engineering Design of Biogas Units For Developing Countries. In: Biogas Technology, Transfer and Diffusion. Elsevier Science Publishing Co., Inc., New York.

59. Contois, D.E. (1959) Kinetics of Bacterial Growth: Relationships Between Population Density and Specific Growth Rate of Continuous Cultures. Journal of General Microbiology 21: 40.

60. Jowel, W.J. (1982) The Feasibility of Biogas Production on Farms. Department of Agricultural Engineering, Cornell University, Ithaca, New York.

61. Hashimoto, A.G. (1981) Ultimate Methane Yield From Beef Cattle Manure. Effect of Temperature, Ratio Constituents, Antibiotics and Manure Age. Agricultural Wastes 3: (4) 241-256.

62. Hashimoto, A.G., and Chen, R.C. (1978) Anaerobic Fermentation of Beef Cattle Manure. Report to the United States Department of Energy.

63. Chica, G. (1983) Biogas Production From Banana Residues (In Spanish). National University of Colombia. Colombia, Medellin.

64. Owen, W.F., Stuckey, D.C., Healy, J.B. Jr., Young, L.Y., and McCarty, P.L. (1979) Bioassay for Monitoring Biochemical Methane Potential and Anaerobic Toxicity. Water Research 13: 485-492.

65. Tewari, H.K., Marwaha, S.S., and Rupal, K. (1986) Ethanol From Banana Peels. Agricultural Wastes 16: 135-146.

66. HACK. Jirka, A.M., and Carter, M.J. (1975) Oxygen Demand, Chemical Range: 0-150 and 0-1,500 mg/L Reactor Digestion Method. Analytical Chemistry 47: (8) 1397-1401.

67. Standard Methods For the Examination of Water and Wastewater (1985) 16th edn. American Public Health Association, American Water Works Association and Water Pollution Control Federation.

68. Hashimoto, A.G. (1989) Effect of Inoculum/Substrate Ratio on Methane Yield and Production Rate From Straw. Biological Wastes 28: 247-255.

69. Basic Biotechnology (1987) Bullock, John and Kristiansen, Bjorn (Eds). Academic Press, New York.

70. Basaraba, J. (1966) Effects of Vegetable Tannins on Glucose Oxidation by Various Microorganisms. Canadian Journal of Microbiology 12: 787-792.

71. El-Gohary, F.A., Nasr, F.A., and Alv, O.A. (1986) The Toxicity Effect of Pesticides and Herbicides on The Anaerobic Digestion Process. In: Biogas Technology, Transfer and Diffusion. Elsevier Science Publishing Co., Inc., New York.

BIOGRAPHICAL SKETCH

Carlet Saint-Phard was born on April 25, 1952, in Port-au-Prince, Haiti. He attended Petit Séminaire Collège Saint-Martial, a Catholic school, from elementary to high school. He received awards for outstanding accomplishment and for outstanding achievement in Spanish. He graduated from high school in 1971. This same year he obtained his diploma of bookkeeper and accountant.

Soon, he realized that food was the bottom line of human activities, that food was basically implicated in the causes of almost all the differences and oppositions of the social classes, and thet food was an integrated system of studies. He decided, then, to become a food expert. What a challenge! The pursuit of this goal led him to the National Autonomous University of Mexico from which he received his bachelor's degree in chemistry, pharmacy, biology with a concentration in food technology in 1981. In 1986, he entered Graduate School at the University of Florida to pursue a Master of Science degree program with a major in food science and human nutrition and a minor in food and resource economics. He expects to graduate

in August 1990. Now, after several years as a full-time student, he feels that he is better equipped to confront the real world where he will just begin to learn.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a thesis for the degree of Master of Science.

Ross D. Brown, Jr., Chair
Associate Professor
Department of Food Science and Human Nutrition

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a thesis for the degree of Master of Science.

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